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Reveiw Article

Biosurfactant-producing Microorganisms: Potential for Bioremediation of Organic and Inorganic Pollutants

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ABSTRACT

The contamination of soil and water by heavy metals and hydrophobic organic compounds poses a significant threat to the environment. Traditional physicochemical methods for remediation are often expensive and environmentally unfriendly, while bioremediation offers a more eco-compatible and economically feasible alternative. Bioremediation utilizes microorganisms, plants, or microbial/plant enzymes to detoxify contaminants in various environments. Biosurfactants, amphiphilic compounds produced by microorganisms, play a crucial role in enhancing bioremediation effectiveness. They increase substrate surface area, create microenvironments, and promote emulsification, thereby facilitating the removal of pollutants. This article provided a comprehensive overview of biosurfactant-producing microorganisms and their potential in the bioremediation of organic and inorganic pollutants. The types and classifications of biosurfactants as well as the factors influencing their production were discussed. Various microorganisms, including bacteria, fungi, and yeasts, were identified as biosurfactant producers. This study outlined the production process and highlighted the importance of optimizing growth conditions for high-quality biosurfactant production. The applications of biosurfactants in remediation were explored by emphasizing their ability to enhance biodegradation, remove heavy metals, and increase hydrocarbon bioavailability. Several studies demonstrating the efficacy of biosurfactant-producing microorganisms in bioremediation were presented. The potential limitations and challenges associated with biosurfactant application in situ were also discussed. In conclusion, the controlled use of biosurfactants could offer promising prospects for the efficient and sustainable cleanup of contaminated sites, contributing to environmental remediation efforts.

1. Introduction

Heavy metals, such as Copper (Cu), Cadmium (Cd), Lead (Pb), Nickel (Ni), Chromium (Cr), and Zinc (Zn), as well as hydrophobic organic compounds cause soil and water contaminations, leading into a serious environmental problem[1,2].

Several different physicochemical and biological processes are employed to cleanup heavy metals and organic compounds from environment[3,4]. Physicochemical methods are not cost-effective, and some of them are not environmentally friendly, while bioremediation processes are an eco-compatible and economically feasible option[2,3].

Bioremediation is the process of using microorganisms, plants, or microbial or plant enzymes to detoxify contaminants in the soil, water, air, flue gases, industrial effluents, and other environments[5]. The natural ability of organisms to adsorb, accumulate, and degrade common and emerging pollutants has attracted the use of biological resources in treatment of contaminated environment[3]. Bioremediation can be done *in situ* at the site of the contamination or *ex situ* away from the site[5].

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Bioremediation can be improved by the use of biosurfactants produced by microorganisms. Biosurfactants can improve bioremediation effectiveness by raising the surface area of substrates, creating their own micro-environment, and promoting emulsification by the assert of certain compounds through diverse mechanisms such as quorum sensing[6].

The aim of the present study was to evaluate a comprehensive overview the biosurfactant-producing microorganisms and their potentiality in bioremediation of organic and inorganic pollutants contaminated sites.

2. Biosurfactants

Biosurfactants are amphiphilic compounds with surface tension reduction abilities that produced by microorganisms such as bacteria, fungi, and yeast[7]. This compound can be used in environmental, industrial, agricultural, therapeutic activities and can also enhance petroleum bioremediation[7].

Biosurfactants have several advantages over chemical surfactants. For instance, biosurfactants are easily degraded by microorganisms in the environment, making them a more sustainable alternative to synthetic surfactants[7]. They are generally non-toxic to humans, animals, and the environment, making them safer to use in various applications[6]. Biosurfactants can lower the surface tension of liquids, which allows them to penetrate and remove dirt and other contaminants more effectively than water alone[6]. In addition, biosurfactants can form stable emulsions of immiscible liquids, making them useful in various industrial applications, such as in the production of food, cosmetics, and pharmaceuticals[2]. Some biosurfactants have been found to have antimicrobial properties, making them useful in the development of new antibiotics and other antimicrobial agents[8]. Biosurfactants can exhibit high selectivity towards different types of hydrophobic compounds, allowing them to be used in specific applications[2,6].

3. Factors for biosurfactant production

The production of biosurfactants by microorganisms is a complex process influenced by several factors, including the type of microorganism, carbon and nitrogen sources, carbon to nitrogen ratio, salinity, temperature, pH, oxygen availability, vitamins, metabolic regulators, inhibitors, inducers, minerals, water and agitation, and aeration[9]. Different microorganisms produce different types of biosurfactants, and the type and concentration of carbon and nitrogen sources used affect biosurfactant production[10]. The optimum temperature, pH, and oxygen levels required for biosurfactant production can vary depending on the microorganism and the biosurfactant being produced[9]. Agitation and aeration of the growth medium are also important for promoting microbial growth and biosurfactant production[11]. Optimizing these factors is crucial for achieving high yields and quality of biosurfactants, which are necessary for their commercial production and various applications[9,10].

4. Types and classifications of biosurfactants

Biosurfactants can be classified into several types based on their chemical structure, microbial origin, and properties[12]. Some biosurfactants may have multiple properties, making them useful in a variety of applications, including in the food, pharmaceutical, and bioremediation industries[12]. Biosurfactants are categorized into glycolipids, lipopeptides, phospholipids, polymeric biosurfactants, fatty acids, and saponins. Glycolipids are biosurfactants that contain a sugar molecule attached to a lipid. Examples include rhamnolipids, sophorolipids, and trehalose lipids[13]. Lipopeptides are biosurfactants that contain a peptide attached to a lipid. Examples include surfactin, iturin, and fengycin[14]. Phospholipids are biosurfactants that contain a phosphate group attached to a lipid. Examples include phosphatidylcholine and phosphatidylethanolamine[13]. Polymeric biosurfactants, including exopolysaccharides and lipopolysaccharides, are produced by microorganisms as part of their extracellular polymeric substances[15]. Fatty acids, such as rhamnolipid fatty acids and sophorolipid fatty acids, are biosurfactants that are produced by microorganisms as a byproduct of their metabolism[12]. Saponins are biosurfactants that are produced by plants and some microorganisms. Examples include glycyrrhizin and aescin[16].

5. Biosurfactant-producing microorganisms

Biosurfactants are produced by a wide range of microorganisms, including bacteria, fungi, and yeasts. Different types of microorganisms belonging to the genera *Pseudomonas*, *Acinetobacter*, *Bacillus*, Candida, *Rhodococcus* and *Corynebacterium*, are reported to produce biosurfactants[1,17]. As indicated by various studies, some potential microbial strains produce biosurfactants (Table 1).

Table 1. Biosurfactant-producing microorganisms

Microorganism	Biosurfactant	Reference
Pseudomonas aeruginosa	Rhamnolipids	18
Torulopsis bombicola, T. apicola	Sophorolipids	19
Rhodococcus erythropolis, Mycobacterium sp	Trehalolipids	20
Bacillus licheniformis	Peptide-lipid	21
Pseudomonas fluorescens	Viscosin	22
Serratia marcenscens	Serrawettin	23
Bacillus subtilis	Surfactin	24
Bacillus subtilis	Subtilisin	24
Bacillus brevis	Gramicidin	25
Mycobacterium sp.	Trehalolipids	23
Corynebacterium lepus	Fatty acid	26
Nocardia erythropolis	Neutral lipids	23
Thiobacillus thiooxidans	Phospholipids	25
Acinetobacter calcoaceticus	Emulsan	25
Acinetobacter calcoaceticus	Biodispersan	25
Candida lipolytica	Liposan	23
Pseudomonas fluorescens	Carbohydrate-lipid-protein	25
Candida tropicalis	Mannan-lipid-protein	23

6. Production of biosurfactants

The production process involves various stages, including selecting a suitable microorganism with the required biosurfactant production capacity, growing an inoculum, preparing a nutrient-rich medium for the production culture, optimizing growth conditions, extracting the biosurfactant, purifying and characterizing the product, and finally, scaling up the process for commercialization[27]. Each step requires optimization of different factors to achieve high yields and quality of biosurfactants, which are vital for their commercial production and use in various applications[27]. Techniques, such as solvent extraction, precipitation, and membrane filtration, are used to extract the biosurfactant from the production culture broth and different characterization techniques are used to determine its properties and structure[28]. Ultimately, the biosurfactant production process must be scaled up while optimizing various parameters to achieve high-quality, cost-effective biosurfactants that meet the needs of different industries[27,28].

7. Applications of biosurfactant in the remediation

Biosurfactants have numerous commercial applications in various industries as a result of their unique properties and eco-friendly nature[6, 23].

Biosurfactants can increase the hydrophobicity of bacterial cells by causing the cell surface to become more hydrophobic[2]. This can increase the association of the cell with the slightly soluble substrate, thereby increasing the bioavailability of the substrate for microbial degradation[23].

Biosurfactants play a crucial role in raising the surface areas of insoluble compounds by reducing surface and interfacial tensions. This, in turn, enhances the mobility and bioavailability of hydrocarbons[14]. As a result, biosurfactants contribute to the improved bioremidation and removal of hydrocarbons. When biosurfactants are added, it is anticipated that they will enhance hydrocarbon biodegradation through mechanisms, such as mobilization, solubilization, or emulsification[25].

Soil biodegradation techniques benefit from biosurfactant-producing microorganisms for oil spill remediation, the removal of heavy metals, conversion of mousse oil into an oil-in-water emulsion and water, soil washing, and waste treatment[26].

The capability of biosurfactant-producing microorganisms to increase bioremediation rates was reported by numerous studies. The reviewed cases of bioremediation using biosurfactants producing microorganisms are presented in Table 2.

The incorporation of surfactants into contaminated soil has the potential to reduce interfacial tension, resulting in increased mass transfer of contaminants[39]. Numerous studies have demonstrated that different surfactants can effectively enhance processes, such as desorption, solubilization, biodegradation of organic compounds, and removal of heavy metals from soil[2].

Table 2. Biosurfactants producing microorganisms and uses in the bioremediation of oil-contaminated environments.

Microorganisms	Type of biosurfactant	Applications	Reference
Nocardiopsis alba MSA10	Lipopeptide	Bioremediation	24
Rhodococcus erythropolis 3C-9	Glucolipid and trehalose lipid	Oil spill removal operations	29
Candida sphaerica UCP0995	Biosurfactant extract	Remove of Zn Fe Pb	30
Pseudomonas aeruginosa S2	Rhamnolipid	Oil spill removal operations	18
Pseudomonas aeruginosa BS20	Rhamnolipid	Bioremediation of oil pollution	31
Candida tropicalis	Biosurfactant extract	Zn Cu Pb	32
Brevibacillus brevis BAB-6437	Lipopeptide	Cr Azulene	33
Bacillus tequilensis CH	Cyclic lipopeptide (CL)	$CdCl_2$	34
Bacillus *subtilis* BS5	Lipopeptide	Bioremediation of hydrocarbon-contaminated sites	24
Candida sphaerica UCP0995	Protein-carboydrate-lipid complex	Biodegration of oil-contaminated environments	25
Candida glabrata UCP1002	Protein-carboydrate-lipid complex	Oil removal	25
Candida lipolytica UCP0988	Sophorolipids	Oil removal	25
Candida guilliermondii UCP0992	Glycolipid complex	Removal of petroleum derivate motor oil from sand	25
Candida lipolytica UCP0988	Sophorolipids	Removal of petroleum and motor oil adsorbed to sand	25
Rhodococcus wratislaviensis BN38	Glycolipid	Bioremediation	37
Pseudozyma hubeiensis	Glycolipid	Bioremediation of hydrocarbon-contaminated sites	35
Pseudomonas cepacia CCT6659	Rhamnolipid	Bioremediation of marine and soil environments	38
Pseudomonas aeruginosa S2	Rhamnolipid	Environmental applications	23
Rhodococcus sp. TW53	Lipopeptide	Bioremediation of marine oil pollution	25
Pseudomonas aeruginosa BS20	Rhamnolipid	Bioremediation of hydrocarbon-contaminated sites	25
Micrococcus luteus BN56	Trehaloset etraester	Biodegration of oil-contaminated environments	36

Parhamfar et al. reported Alcanivorax and Idiomarina had the highest capacity to produce biosurfactant and remove approximately 90% and 53% of crude oil, respectively[40]. In another study, it was found that Exopolysaccharide (EPS) secreted by Enterobacter cloacae strain TU was as an emulsifier with a high emulsifying activity (E24 = 75)[15].

Rhamnolipids have been suggested as effective agents for soil washing, aiming to enhance the removal of organic pollutants and metals from soil[38]. However, there is a potential limitation to their application due to sorption by soil matrix components. *Pseudomonas* spp. are capable of producing rhamnolipids, either in the form of monorhamnolipids or a combination of mono- and dirhamnolipids[38]. The sorption of monorhamnolipids on soil matrix components depends on its concentration[41]. Monorhamnolipid form exhibits stronger sorption when present alone compared to when it is in a mixture of forms[41].

Conte et al. performed a study to compared the efficiency of a humic acid solution to that of common surfactants, such as sodium dodecyl sulfate (SDS) and Triton X-100 (TX100), as well as water, in the washing of polluted soil in a contaminated industrial area near a chemical plant[42]. The results revealed that water alone was unable to completely remove pollutants from the soil. On the other hand, all the organic surfactants exhibited similar efficiencies, achieving pollutant removal rates of up to 90%. Consequently, the use of natural humic acid solutions appears to be a more favorable option for soil washing in highly contaminated areas. This is due to their additional capacity to promote microbial activity, which is in contrast to chemical surfactants commonly used for soil washing[42].

Biosurfactants could potentially be utilized to decrease metal contamination[30]. Unlike biodegradable substances, heavy metals cannot be broken down by biological processes. Instead, they can undergo transformations from one chemical state to another, altering their mobility and level of toxicity[32].

Microorganisms have the ability to impact metals through various mechanisms. One way is through the transformation of metals via redox processes or alkylation[43]. Additionally, microorganisms can accumulate metals through either passive uptake, which occurs independently of metabolism, or active uptake. This involves intracellular processes that depend on metabolism[44]. Furthermore, microorganisms can indirectly influence the mobility of metals by altering pH levels or by producing and releasing substances that affect the mobility of these metals[45].

Due to their anionic nature, Rhamnolipids enable effectively extract metals and ions, including cadmium, copper, lanthanum, lead, and zinc, from soil. This ability can be attributed to their capacity for complexation[46]. Rhamnolipids have the potential to remove heavy metals from sediments. When the biosurfactant is utilized in a continuous flow configuration, it achieves a removal efficiency of up to 37% for copper, 13% for zinc, and 27% for nickel[47].

In another study, the removal of cadmium using an aqueous solution was observed even at concentrations below the critical micelle concentration (CMC). Furthermore, when the concentration of the solution was five times higher than the CMC, it led to the nearly complete removal of 100 ppm of metallic ions[48].

Biosurfactants offer potential applications for the removal of metals from soil[38]. One method involves applying biosurfactants to a specific section of contaminated soil, where the soil is placed in a large cement mixer. Through this process, the biosurfactant-metal complex is flushed out, allowing for separation. The soil is then returned to its original location, while the biosurfactant-metal complex is treated to precipitate out the biosurfactant. This separation process leaves behind the metals, which can be further addressed or managed separately[49,50]. The strong bond between the positively charged metal and the negatively charged surfactant enables the effective removal of the surfactant-metal complex from the soil matrix through the flushing process[26]. This method is particularly suitable for surface-level contamination, where water is flushed through the soil to remove the complex. However, for deeper subsurface contamination, additional pumping activities may be necessary to carry out the process effectively[26,49].

The utilization of biosurfactants offers undeniable advantages in the context of heavy metal-contaminated soil, as bacterial strains capable of producing surface active compounds do not necessarily require survival ability in such environments[49]. However, the use of biosurfactants alone necessitates the continuous addition of new portions of these compounds[49].

The biosurfactant can positivily affect the bioremediation of heavy metal-contaminated soil due to their capacity to create metal-based complexes[50]. Anionic biosurfactants form metal-based complexes in a nonionic form using ionic bonds[50]. Compared to the metal's interactions with the soil matrix, the bonds are stronger, which lead to the desorption of metal-biosurfactant complexes from the soil matrix into the soil solution.

Although using biosurfactants in bioremediation is highly effective, there are still many factors that needs further investigations. Despite positive effects of biosurfactants, there are some cases for which no or negative effects were recorded. This discrepancy may be explained by different conditions uderwhich the studies were performed, that is the laboratory and *in situ* conditions.

8. Conclusion

Biosurfactants hold significant potential for remediating contaminated sites. They can be effectively employed to treat soil and water that have been contaminated with both organic and inorganic pollutants. By employing biosurfactants in a careful and controlled manner, the cleanup of toxic environmental pollutants can be enhanced, leading to a cleaner and healthier environment. Biosurfactants offer a promising solution for

the bioremediation of contaminated sites. These compounds, produced by microorganisms, possess unique properties that make them effective in removing organic and inorganic pollutants from soil and water. Biosurfactants have diverse applications in industry, including enhanced biodegradation of hydrocarbons, soil washing, dispersing oil slicks, oil spill remediation, water and waste treatment, and metal removal. They can increase the bioavailability of contaminants, enhance biodegradation rates, promote solubilization and emulsification of hydrocarbons, and form complexes with heavy metals for their removal from soil and water. Despite the promising potential of biosurfactants, further research is needed to fully understand their effects under different conditions and *in situ* applications. Consistency between laboratory studies and field trials is essential to validate their effectiveness and optimize their use in real-world scenarios.

Declarations

Competing interests

The authors declare that they have no conflict of interest.

Authors' contribution

All authors were involved in data collection, design of the article, interpretation of results, review, and manuscript preparation.

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Ethical considerations

The authors checked for plagiarism and consented to the publishing of the article. The authors have also checked the article for data fabrication, double publication, and redundancy.

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